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LETTER TO THE EDITOR

Donor-Specific Mononuclear Cell Transfusion and Methotrexate as Pretransplant Treatment in Dogs Given DLA-Identical Marrow Grafts after Nonmyeloablative Conditioning

We previously reported stable engraftment in dogs given 2 Gy total body irradiation (TBI) before and short courses of immunosuppression with cyclosporine and mycophenolate mofetil [1] or cyclosporine and rapamycin after DLA-identical marrow transplantation [2]. When the TBI dose was lowered to 1 Gy, uniform graft rejection was seen [1,2].

Stable grafts were also seen in dogs given 4.5 Gy radiation to the cervical, thoracic, and upper abdominal lymph node chains [3] and in those that received, in addition to 1 Gy TBI, injections of both donor-derived peripheral blood mononuclear cells (PBMCs) and the T-cell activation blocker CTLA4-immunoglobulin [4]. Results were consistent with the notion that induction of immunologic host versus donor hyporesponsiveness before and administration of a short course of immunosuppression after hematopoietic cell transplantation sufficed to establish long-term marrow engraftment.

In the present study, we substituted the antimetabolite methotrexate (MTX) for CTLA4-immunoglobulin to induce host-versus-graft hyporesponsiveness under the assumption that host T cells that proliferate in response to donor PBMCs would be especially sensitive to MTX. For many years, MTX has been an integral part of graft-versus-host disease prevention [5-7], and it has been shown to promote activation-induced cell death [8].

Litters of beagles, mini-mongrel, and golden retriever crossbreeds weighing 7.2-11.3 kg were used. Details on vaccinations, selection of littermate donor-recipient pairs by DLA typing, marrow harvest and injection, postgrafting care, and assessment of engraftment have been described [1,2]. The study was approved by the Institutional Animal Care and Use Committee at the Fred Hutchinson Cancer Research Center (Seattle, WA), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

On days −5 and −3 (day 0 was the day of marrow transplantation), dogs received donor PBMCs (5×10^6 cells/kg/d intravenously, except for G477 (1×10^7 cells/kg on day −5 only). On days −4 and −2, 24 hours after each PBMC injection, MTX (.4 mg/kg/d) was administered intravenously. On day 0, recipients were given 1 Gy of TBI at 7 cGy/min from a linear accelerator (CLINAC 4, Varian, Palo Alto, CA). Marrow was infused intravenously within hours of TBI (Table 1). Dogs were given cyclosporine, 15 mg/kg twice daily orally, on days −1 to 35 and mycophenolate mofetil, 10 mg/kg twice daily subcutaneously, on days 0 to 27.

Peripheral blood cell changes included moderate thrombocytopenia, neutropenia, and lymphocytopenia (Figure 1; nadir range, days 3-27). Only 1 dog required a platelet transfusion. There were no infections and no graft-versus-host disease. All 6 dogs had initial engraftment (Table 1), with maximum donor chimerism levels ranging from 2% to 70% (granulocytes) and 2% to 49% (lymphocytes), respectively. Eventually 4 of the 6 dogs rejected their grafts be-

Table 1. *Marrow Grafts from DLA-Identical Donors after Donor PBMC, MTX, and 1-Gy TBI*

Dog No.	Marrow Cells (10^8/kg)	Maximum Donor Chimerism, %		Graft Rejection	Duration of Engraftment (wk)
		Granulocytes	Mononuclear Cells		
G231	7.00	21	23	Yes	10
G254	2.47	28	23	Yes	8
G269	2.16	2	2	Yes	5
G281	2.05	45	32	No	>26
G322	7.23	70	47	No	>26
G447	3.16	46	49	Yes	8

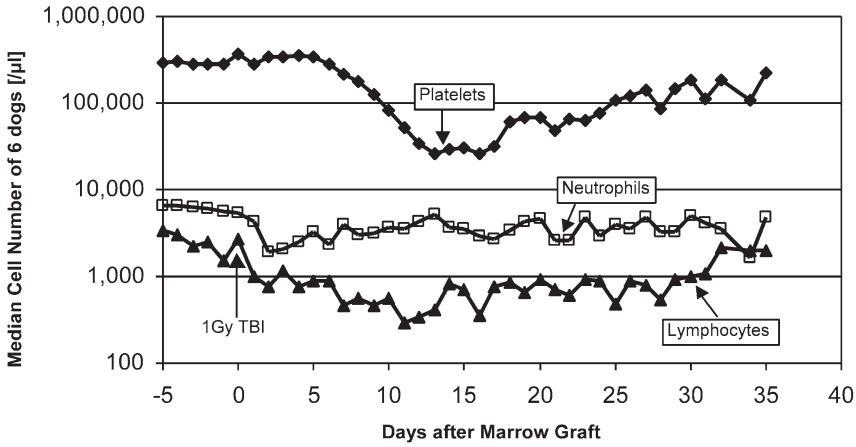

Figure 1. Median peripheral blood changes in 6 dogs given donor-specific transfusion, MTX, and 1 Gy TBI before and mycophenolate mofetil/cyclosporine after marrow grafts from DLA-identical littermates.

tween 5 to 10 weeks, whereas 2 dogs had sustained engraftment.

Compared with historical controls given neither donor PBMCs nor MTX [1,2], median duration of engraftment in the 6 dogs (9 weeks; $P = .3384$, log-rank test) and rate of sustained engraftment ($P = .4545$, Fisher exact test) showed no statistically significant difference.

In conclusion, 4 of 6 dogs rejected their marrow grafts, as evidenced by decreasing donor chimerism levels, within a time frame that was similar to that seen in previously published controls [1,2], and only 2 of the 6 had sustained engraftment. Clearly, the degree of host-versus-graft hyporesponsiveness achieved with MTX was less than previously accomplished with T-cell costimulatory blockade through CTLA4-immunoglobulin [4]. Whether MTX will be useful when combined with T-cell costimulatory blockade or immunosuppressive drugs with a different mechanism of action, for example, rapamycin, remains to be investigated.

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